

Novel Dextranucrase Gtf-DSM, Highly Similar in Sequence to Reuteransucrase GtfO, Displays Unique Product Specificity

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Figure S1. The SEC profile of recombinant Gtf-DSM. The main peak ranging from 68 to 78 min corresponds to a monomeric protein according to the elution time and determined standard proteins. The target protein was collected within this main peak.

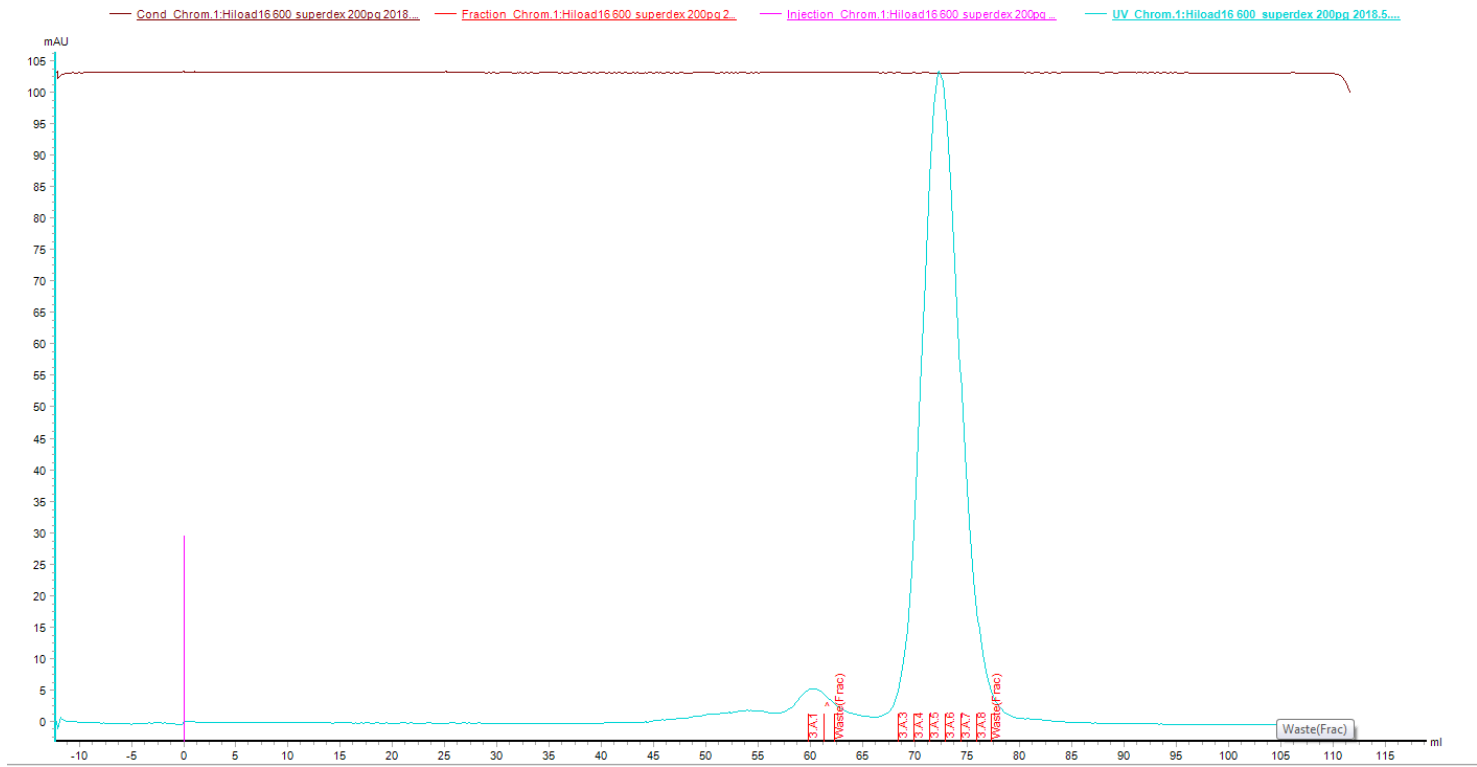


Figure S2. Amino acid sequence alignment of conserved motifs I (green), II (raspberry), III (blue) and IV (yellow) of Gtf-DSM and reported GS enzymes. The strictly conserved residues are highlighted by a red background. The acceptor binding subsites -1, +1 and +2 of the resolved Gtf180 enzyme are correspondingly labelled at the bottom of motifs. NU, nucleophile; A/B, general acid/base; TS, transition state stabilizer.

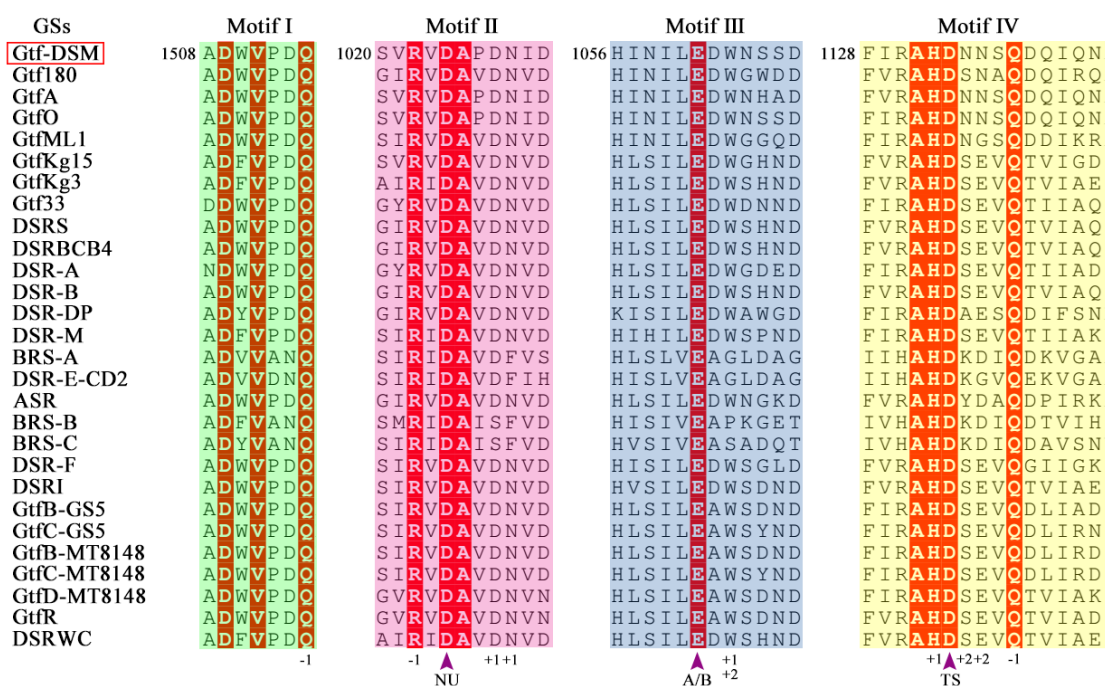


Figure S3. Amino acid sequence alignment of twelve residues of native Gtf-DSM with that of other reported GS enzymes.

	739	758	765	768	889	937	977	1083	1086	1087	1458	1529
GTF-DSM	L	V	Q	D	S	I	D	D	Q	K	D	V
GTFO	F	G	H	Y	N	L	A	V	K	G	A	G
GTF180	F	P	Q	N	N	L	A	D	R	N	D	S
GTFA	F	P	Q	N	N	I	Q	D	K	N	D	V
GTFML1	F	P	Q	N	N	L	S	Y	S	T	D	V
GTFKg15	T	L	I	N	N	D	Y	D	H	T	D	T
GTFKg3	T	M	S	D	T	D	Y	D	H	T	D	T
GTF33	S	V	I	G	T	D	Y	F	H	L	D	T
DSRS	T	L	V	G	N	D	F	D	H	T	D	T
DSRBCB4	I	L	V	G	N	D	F	D	H	T	D	T
DSR-A	P	P	I	G	T	D	F	F	H	L	D	T
DSR-B	I	L	V	G	N	D	F	D	H	T	D	T
DSR-DP	T	L	I	G	L	D	Y	T	K	D	D	V
DSR-M	T	R	V	N	N	N	Y	A	Q	N	D	T
BRS-A	K	Q	N	G	N	.	L	K	T	N	D	V
DSR-E-CD2	K	Q	I	G	N	A	F	L	T	N	D	A
ASR	T	L	T	N	N	Q	S	Y	T	S	D	V
BRS-B	K	Q	N	G	N	.	L	Q	S	K	G	V
BRS-C	K	Q	N	G	N	.	M	N	S	K	G	V
DSR-F	K	L	T	N	N	Q	S	T	Q	N	D	V
DSRI	K	V	V	G	N	D	Y	N	R	L	D	V
GTFB-GS5	P	V	I	G	N	D	Y	N	R	L	D	V
GTFC-GS5	K	L	N	G	N	D	Y	N	R	L	D	V
GTFB-MT8148	P	V	I	G	N	D	Y	N	R	L	D	V
GTFC-MT8148	K	L	N	G	N	D	Y	N	R	L	D	V
GTFD-MT8148	P	P	T	N	N	D	Y	N	R	L	D	V
GTFR	S	V	E	G	T	D	Y	N	R	L	D	V
DSRWC	T	M	S	D	T	D	Y	D	H	T	D	T
	N-ter	Domain V		Domain IV	Domain B		Domain A			Domain B		

Figure S4. Nano DSC analysis of the recombinant Gtf-DSM.

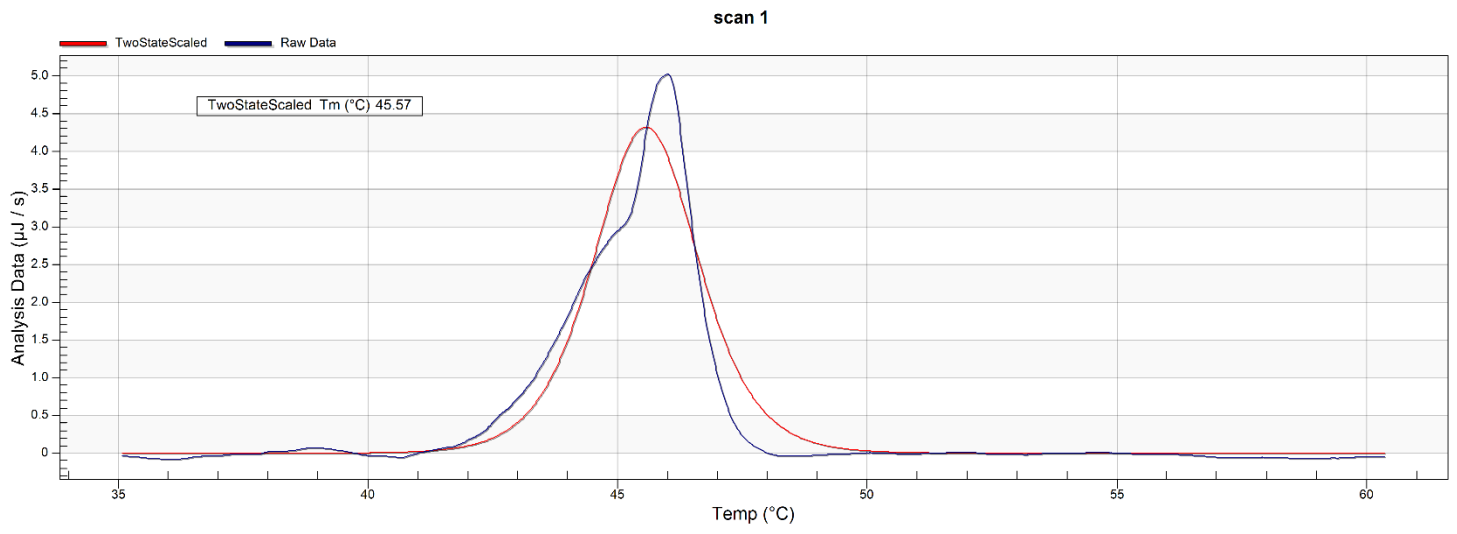


Figure S5. The HPSEC analysis with RI and MALLS detectors for the dextran produced by the recombinant Gtf-DSM. The differential refractive index and molecular weight distribution were represented by blue and red lines respectively.

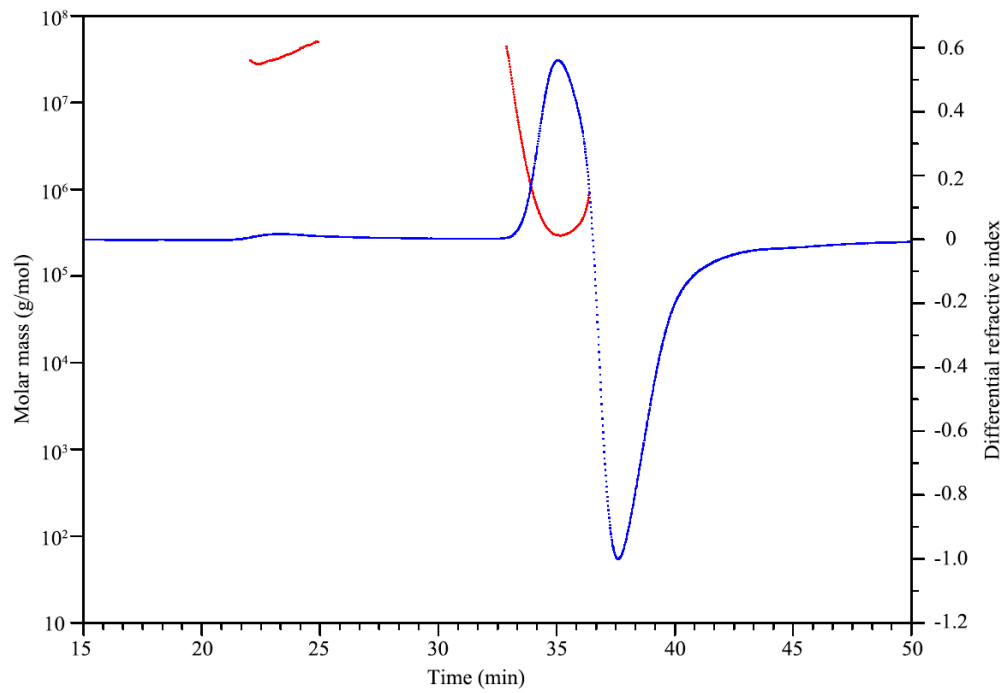


Table S1. Comparison of amino acid sequence homology of the Gtf-DSM and reported GS enzymes from various microorganisms.^a

GS enzymes	Identity (%)
Gtf-DSM	100
Gtf180	60.91
GtfA	69.72
GtfO	99.33
GtfML1	58.12
GtfKg15	40.49
GtfKg3	42.18
Gtf33	43.64
DSRS	43.55
DSRBCB4	42.11
DSR-A	45.16
DSR-B	44.38
DSR-DP	37.66
DSR-M	38.88
BRS-A	40.3
DSR-E-CD2	45.39
ASR	41.72
BRS-B	38.07
BRS-C	37.42
DSR-F	42.11
DSRI	43.91
GtfB-GS5	44.04
GtfC-GS5	43.87
GtfB-MT8148_Gtf-I_	44.64
GtfC-MT8148_Gtf-SI_	42.36
GtfD-MT8148_Gtf-S_	42.58
GtfR	40.09
DSRWC	42.23

^a Gtf180 (*L. reuteri* 180), GtfKg15 (*Lactobacillus sakei* Kg15), GtfKg3 (*Lactobacillus fermentum* Kg3), Gtf33 (*Lactobacillus parabuchneri* 33), DSRS (*L. mesenteroides* NRRL B-512F), DSRBCB4 (*L. mesenteroides* B-1299 CB4), DSR-A (*L. citreum* NRRL B-1299), DSR-B (*L. citreum* NRRL B-1299), DSR-DP (*L. citreum* NRRL B-1299), DSR-M (*L. citreum* NRRL B-1299), DSR-F (*L. citreum* B/110-1-2), DSRWC (*Weissella cibaria* CMU), GtfR (*Streptococcus oralis* ATCC10557), GtfA (*L. reuteri* 121), GtfO (*L. reuteri* ATCC 55730), GtfML1 (*L. reuteri* ML1), DSRI (*L. mesenteroides* NRRL B-1118), GtfB-GS5 (*S. mutans* GS5), GtfC-GS5 (*S. mutans* GS5), GtfB-MT8148 (*S. mutans* MT8148), GtfC-MT8148 (*S. mutans* MT8148), GtfD-MT8148 (*S. mutans* MT8148), ASR (*L. mesenteroides* NRRL B-1355), DSR-E CD2 (*L. citreum* NRRL B-1299), BRS-A (*L. citreum* NRRL B-1299), BRS-B (*L. citreum* NRRL B-742), BRS-C (*L. fallax* KCTC3537).